

# 사람 코점막과 배양된 코점막 상피세포에서 Nonsteroidal Anti-Inflammatory Drug-Activated Gene-1 (NAG-1)의 발현

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## Expression of Nonsteroidal Anti-Inflammatory Drug-Activated Gene-1(NAG-1) in Human Nasal Mucosa and Cultured Nasal Epithelial Cells

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### ABSTRACT

**Background and Objectives** : Nonsteroidal anti-inflammatory drug-activated gene-1 (NAG-1) is a recently discovered TGF- $\beta$  superfamily cytokine. But localization and functions of NAG-1 have not been thoroughly studied. So, we wanted to investigate its expression and localization in human nasal mucosa and also wanted to investigate the change of NAG-1 expression as a function of mucociliary and squamous differentiation. **Materials and Method** : Anterior and middle portion of human inferior turbinate were used and immunohistochemistry with NAG-1 antibody was done. Passage-2 normal human nasal epithelial cell culture using air-liquid interface method was performed for 14 days and the cells were divided as retinoic acid (RA)-sufficient and RA-deficient group. Hematoxylin and eosin staining was done on each group to study the degree of differentiation. Western blot analysis for NAG-1 expression was performed on each group on 0, 7, and 14 days. **Results** : NAG-1 expression of mucociliated epithelium was noted in ciliated cells and serous acini, but was not found in goblet cells and mucous acini. In the squamous epithelium, its expression was weaker than in the mucociliated epithelium. In the RA-sufficient culture, NHNE cells were differentiated into ciliated epithelium, but in the RA-deficient culture, keratinizing squamous epithelium was noted. In the Western blot analysis, NAG-1 expression was significantly higher in the RA-sufficient culture than in the RA-deficient culture and this expression was time-dependent. **Conclusion** : NAG-1 may be related to differentiation and apoptotic process of nasal epithelial cells. However, it is still unclear whether NAG-1 is an inducer or a byproduct of differentiation or apoptosis. The role of NAG-1 protein remains to be solved. (Korean J Otolaryngol 2003;46:396-400)

**KEY WORDS** : Nasal mucosa · Anti-Inflammatory agents, Non-Steroidal · Cell differentiation.

Nonsteroidal anti-inflammatory drug-activated gene-1(NAG-1)  
 TGF- $\beta$  superfamily 가 TGF- $\beta$  superfamily  
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.<sup>1)</sup> NAG-1 placenta bone morpho-  
 genic protein,<sup>2)</sup> placenta TGF- $\beta$ ,<sup>3)</sup> prostate derived  
 factor,<sup>4)</sup> macrophage inhibitory cytokine-1<sup>5)</sup> novel  
 TGF- $\beta$  superfamily HP00269<sup>6)</sup>  
 가  
 TGF- $\beta$  superfamily  
 .<sup>1)</sup>  
 TGF- $\beta$  superfamily  
 , ,  
 ,<sup>7)</sup>  
 G1 ,

<sup>8)</sup> NAG - 1 (CA, USA) (normal blocking antibody) 20  
 , rabbit anti - NAG - 1 (a generous gift  
 from Dr. Thomas E. Eling : NIEHS, RTP, NC, USA)(1 :  
<sup>1)2)5)</sup> NAG - 1 가 ,<sup>9)</sup> 5,000 ) 4  
 5 3  
 biotinylated goat anti - rabbit IgG 30  
 3,3' - diaminobenzidine substrate kit(Vector  
 Laboratories) 가  
<sup>10)</sup> ,  
<sup>11)</sup> NAG - 1 TGF - superfamily  
 NAG - 1 가 2 Transwell -  
 NAG - 1 가 clear ( 24.5 mm, 0.45 mm pore)(Costa Co. :  
 NAG - 1 Cambridge, MA, USA) 10<sup>5</sup>  
 , , BEGM DMEM  
 (localization) fetal bovine serum  
 가 .<sup>11)</sup> 가 가  
 NAG - 1 가 . All - trans  
 (Sigma Co : St.Louis, MO, USA)  
 10<sup>-7</sup> M . 9  
 NAG - 1 가 Air - liquid interface  
 가  
 NAG - 1  
 air - liquid interface 14  
 10% 2% agarose  
 , 4 μm  
 hematoxylin eosin  
 (Institutional Review Board  
 of Yonsei University College of Medicine)  
 NAG - 1 air -  
 liquid interface , 7 , 14  
 radioimmunoprecipitation assay buffer(1% NP - 40,  
 0.5% sodium deoxycholate, 0.1% SDS)  
 10% 4 20 10,000 ×g  
 4 μm xylene , Western blot  
 3% 10 bovine serum albumin BCA  
 , 2% 0.2% Triton protein assay (30 μm)  
 (phosphate buffered saline) 15% SDS - polyacrylamide gel electrophoresis(PAGE)  
 Vectastain Elite (Amersham Pharmacia Bio-  
 ABC peroxidase kit(Vector Laboratories : Burlingame, tech : Piscataway, NJ, USA) . 10%

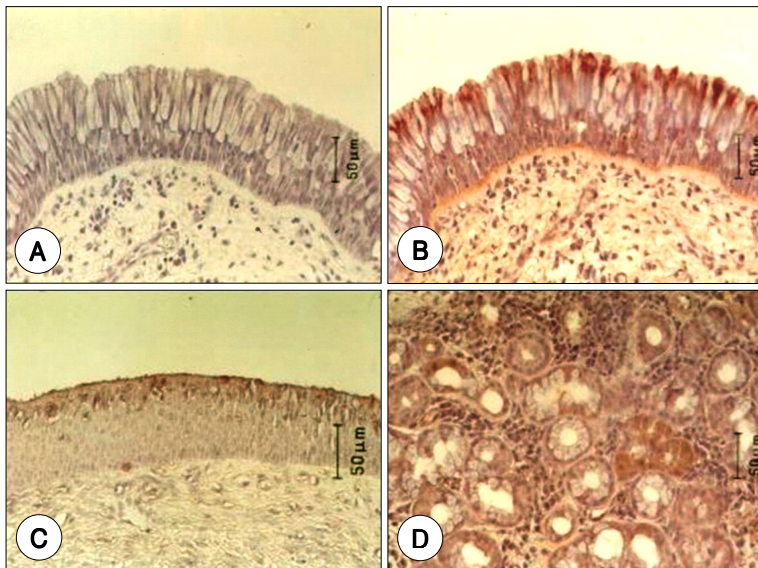
사람 코점막과 배양 세포의 NAG-1 발현

가 TBST (Tris - buffered saline/0.05% Tween - 20) 4 가 , 2% 가 TBST 1 : 5000 anti - NAG - 1 1 : 500 anti - actin (Santa Cruz Biotechnology Inc. : Santa Cruz, CA, USA) 4 HRP - anti - rabbit enhanced chemiluminescence autoradiography

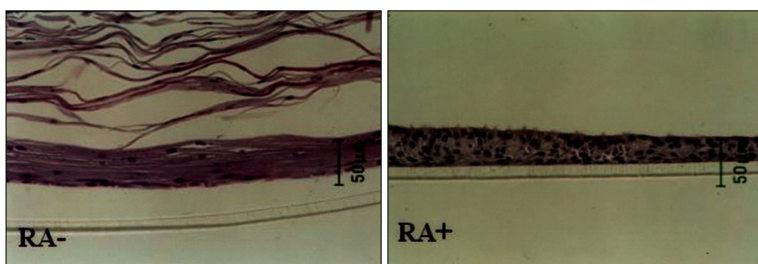
, NAG - 1 NAG - 1 (Fig. 1C). (Fig. 1D). (preimmune serum) (Fig. 1A). 가

가 가 air - liquid interface 14 가 hematoxylin eosin 가 가 (Fig. 2). , NAG - 1 가

(Fig. 1B).

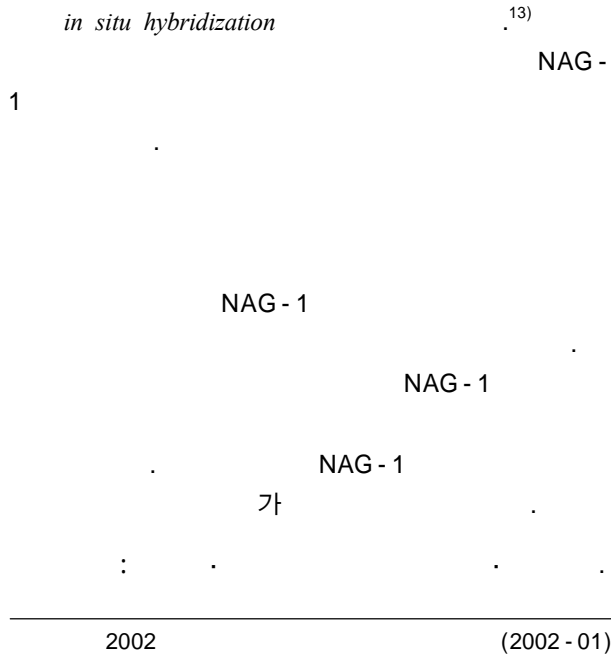


**Fig. 1.** Expression of NAG-1 in human nasal mucosa. A : Negative control in which the preimmune serum was used as primary antibody. B : In mucociliary epithelium, NAG-1 expresses in the upper portion of cytoplasm of ciliated cells, but not in basal and goblet cells. C : In squamous epithelium, NAG-1 expresses only in uppermost cells, but its intensity is weaker than that in ciliated cells. D : In submucous glands, NAG-1 expresses in serous acini, but not in mucous acini.



**Fig. 2.** Histology of normal human nasal epithelial (NHNE) cells in retinoic acid (RA)-sufficient and RA-deficient culture. NHNE cells were cultured with or without RA for 14 days after creating air-liquid interface and were stained using hematoxylin and eosin. Cultured cells differentiates into mucociliated epithelium in RA-sufficient culture (RA+) and keratinizing squamous epithelium in RA-deficient culture (RA-).





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